PCR-derived methods

- **Taq Polymerase (Colony PCR; for fragments shorter than ~1,2 kb)**

Master Mix (50 µl):

	Stock concentration	**Volume**	**Final concentration**
Taq buffer*	10x	5 µl	1x
H_2O	-	39 µl	-
Primer I	10µM (10 pmol/µl)	2 µl	0,4 µM
Primer II	10µM (10 pmol/µl)	2 µl	0,4 µM
dNTPs	10 mM of each	1 µl	200 µM each
Taq polymerase	-	1 µl	-

Programme:

94°C 2:00

94°C 0:30 ┐

X°C 0:30 ├ 35x

72°C Y:YY ┘

72°C 10:00

15°C ∞

X°C – annealing temperature

Y:YY – extension time (1 min/kb)

- **Q5 High-Fidelity DNA Polymerase from NEB**

Master Mix (50 µl):

	Stock concentration	**Volume**	**Final concentration**
5X Q5 Reaction Buffer	5x	10 µl	1x
H_2O	-	33,5 µl	-
Primer I	10µM (10 pmol/µl)	2 µl	0,4 µM
Primer II	10µM (10 pmol/µl)	2 µl	0,4 µM
dNTPs	10 mM of each	1 µl	200 µM each
template	-	1 µl	-
Q5 High-Fidelity DNA Polymerase	2 U/µl	0,5 µl	0,02 U/µl
5X Q5 High GC Enhancer (optional)	5 x	(10 µl)	1x

The 5X Q5 Reaction Buffer provided with the enzyme is recommended as the first-choice buffer for robust, high-fidelity amplification. For difficult amplicons, such as GC-rich templates or those with secondary structure, the addition of the Q5 High GC Enhancer can improve reaction performance. The 5X Q5 Reaction Buffer is detergent-free and contains 2.0 mM $MgCl_2$ at the final (1X) concentration.

Programme:

98°C 0:30

98°C 0:05-0:10 ⎫

X°C 0:10-0:30 ⎬ 35x

72°C Y:YY ⎭

72°C 02:00

4-10°C ∞

X°C – annealing temperature

Y:YY –Extension times are generally 20–30 seconds per kb for complex, genomic samples, but can be reduced to 10 seconds per kb for simple templates (plasmid, *E. coli*, etc.) or complex templates < 1 kb. Extension time can be increased to 40 seconds per kb for cDNA or long, complex templates, if necessary.

Protocol generously provided by the lab
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